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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/992,221
Filing Date: November 06, 2001
Appellant(s): TSUJI ET AL.

Kent H. Cheng
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed May 21, 2004.

(1) Real Party in Interest

A statement identifying the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

(3) Status of Claims

The statement of the status of the claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Invention

The summary of invention contained in the brief is correct.

(6) Issues

The appellant's statement of the issues in the brief is correct.

(7) Grouping of Claims

Appellant's brief includes a statement that claims 1-11 do not stand or fall together and provides reasons as set forth in 37 CFR 1.192(c)(7) and (c)(8).

(8) Claims Appealed

The copy of the appealed claims contained in the Appendix to the brief is correct.

(9) Prior Art of Record

5,298,426	INAMI et al	3-1994
5,559,037	KIM et al	9-1996
4,284,412	HANSEN et al	8-1981
4,492,752	HOFFMAN et al	1-1985
5,516,695	KIM et al	5-1996

BENTLEY, S.A. et al. "Correction of Bone Marrow Nucleated Cell Counts for the Presence of Fat Particles." American Journal of Clinical Pathology, volume 104, no. 1, (July, 1995) pages 60-64.

(10) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claims 1-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Inami et al (US 5,298,426), Kim et al (US 5,559,037), Hansen et al (U.S. 4,284,412), and Hoffman et al (US 4,492,752) in view of Bentley, (American Journal of Clinical Pathology, volume 104, no. 1, (July, 1995) pages 60-64), and further in view of Kim et al (US 5,516,695).

The claims are directed to methods of analyzing bone marrow. The marrow is subjected to analytical steps allowing

for a determination of the various types of cells present in the sample. The total number of cells, as well as the number of specific cell types is of clinical significance, especially in situations where the marrow is to be transplanted. See, e.g., Bentley, introductory paragraphs at page 60.

Inami discloses a method of measuring the number of erythrocytic nucleated cells by the following steps: 1) a sample of blood cells containing erythroblasts is mixed with a hypotonic lysis solution at pH 3.5 to 5.0 to lyse the erythrocytes and further comprising a fluorescent nuclear dye to stain the nucleated cells differentially, and then analyzed by flow cytometric analysis via scattered light and fluorescence. The different nucleated cell types can be distinguished and counted (column 1, line 67 to column 2, line 24). Erythroblasts are normally found in the bone marrow and not in circulating blood, and their differentiation and counting is clinically important in the treatment of certain diseases such as anemia and leukemia (column 1, lines 25-31). Inami also discloses that the analytical methods disclosed therein can be applied to bone marrow samples, as recited in appellant's claims (see column 7, line 65 to column 8, line 12).

Kim et al disclose taking a sample of blood cells, exposing it to an erythrocyte lysing solution further comprising a

nuclear staining dye and analyzing the solution by flow cytometry using scattered light and fluorescence to distinguish and count the different nucleated cell types. A wide range of fluorescent dyes, including dyes recited in the claims under examination, may be used (column 6, lines 18-56).

Hansen et al disclose taking a sample of blood cells, incubating them with a fluorescent antibody specific to a subclass of cell antigens, lysing the erythrocytes in the sample in the conventional fashion, and analyzing the resulting sample by flow cytometry by means of scattered light and fluorescence (column 5, line 40 to column 6, line 34) so as to count the identified cells.

Hoffman et al provides a similar disclosure to Hansen, but wide-angle scattering, recited in the claims under examination, is used.

Thus, Inami, Kim '037, Hansen and Hoffman demonstrate that the claimed dyes were all known to the artisan of ordinary skill to be useful in the analysis of blood cell-containing samples, such as bone marrow, using the claimed analytical parameters of fluorescent and light scattering intensity, as recited in the claims under examination. Inami, Kim '037, Hansen and Hoffman, differ from the claims under examination in that those patents fail to disclose the step of classifying the lipid particles

present in the analyzed marrow sample as part of the step of analysis by fluorescence and scattered light.

However, Bentley clearly discloses that when classifying and counting cells in bone marrow to be used in transplantations, the presence of lipid particles in the bone marrow results in an incorrect count of total nucleated cells. See page 60, abstract and introductory paragraphs. As can be seen in Figures 1 and 2 in Bentley, the particles are clearly delineated from the cells. Figure 2 shows how by rearranging the threshold lines, the fat particles can be assigned to boxes that are easily subtracted from the total nucleated cell (TNC) count. Bentley establishes the importance of classifying the fat particles in a bone marrow sample so that an accurate TNC can be obtained, by compensating for the amount of lipid particles in the sample.

Thus, one of ordinary skill in the art performing the analytical procedures of Inami, Kim '037, Hansen and Hoffman would have been motivated by Bentley to have classified the lipid particles present in the marrow sample, and thereby obtain a more accurate cell count. It is proper to combine Inami, Kim '037, Hansen and Hoffman with Bentley, because all references are directed to solving the same problem -- obtaining accurate TNC cell counts in blood cell-containing samples.

Inami, Kim '037, Hansen, Hoffman and Bentley also differ from the claims in that none of those references disclose the use of the surfactants recited in claim 6 and its dependents. However, Kim '695 disclose a multipurpose reagent system for the rapid lysis of erythrocytes in blood samples to be analyzed by flow cytometry via scattered light and fluorescence. The multipurpose reagent comprises ammonium salts (column 6, lines 50-58), aliphatic aldehyde (column 6, line 59 to column 7, line 3), non-phosphate buffer (column 7, lines 4-20), surface active agents such as saponins (column 7, line 21-58, anticoagulant, nuclear stain or antibody with an osmolarity between about 160 and 310 mOsm/L (column 5, lines 30-45). The reagent is said to completely lyse the erythrocytes while maintaining the white blood cells.

A person of ordinary skill in the art at the time the invention was made would have been motivated to have used the method of Inami using the lysis buffer like that of Kim '695 because the lysis buffer of Kim '695 is said to completely lyse the erythrocytes while being gentle enough on the other cells in the blood sample, even the fragile ones, to maintain their integrity. Kim '037, Hansen, Hoffman and Kim '695 all show that using a combination of scattered light and fluorescence in a flow cytometry method to measure and count cells is well known

in the art. Moreover, the claimed analysis of data demonstrated by the cited prior art to be of clinical significance, said analysis involving calculations of ratios of cell types, would have been considered an obvious manipulation of data known to be of clinical significance, the percentages (i.e. ratios) of the various cell types present in the marrow being indicative of the properties of the marrow. See, e.g., Bentley at page 63, first paragraph of Discussion section.

(11) Response to Argument

Appellant urges initially that it has not been established that the absorption method, impedance method and combination thereof used by Bentley are the equivalents of the combination of the fluorescent method and scattered light method recited in the claims under examination. Rather, urges appellant, the absorption and impedance methods combined in Bentley measure properties distinct from the fluorescence/light scattering methods recited in the claims under examination such that the theoretical and physical bases of the two methods are distinct from each other. Moreover, urges appellant, the fact that the result of the present invention as a whole is different from the Bentley reference clearly shows that the combination of fluorescence and scattered light methods is not an equivalent to Bentley's methods.

Appellant's argument does not demonstrate error. Bentley is not cited for its use of any particular analytical technique, per se. Rather, Bentley is cited for the fact that one practicing the cited prior art methods of cell counting and classification, such as disclosed in Inami et al, would have recognized the desirability of classifying the lipid particles so as to ensure an accurate count of the total amount of cells present in the sample, as well as an accurate count of the various cell types therein. Thus, even assuming the techniques of Bentley would not have been considered equivalent to those of Inami and the other patents, the artisan of ordinary skill would nevertheless have recognized from Bentley the importance of classifying lipid particles in a marrow analysis, so as to ensure an accurate cell count, precisely as recited in appellant's claims.

Appellant further urges certain advantages of the claimed methods over Bentley with respect to the ability to distinguish between certain cell types which are not distinguishable using the methods of Bentley, as evidenced by Fig. 2 of the present application when compared to Figures 1 and 2 of Bentley. However, it is respectfully pointed out that Bentley is cited in combination with other references, and that it is therefore improper to argue Bentley's shortcomings to the exclusion of the

other references cited in the rejection. One cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Thus, Bentley is cited in combination with prior art which discloses the ability of fluorescence and scattered light to distinguish between cell types in bone marrow samples. The ability of the claimed process to distinguish between those cell types cannot therefore be considered unexpected since the prior art cited along with Bentley, e.g. Inami as discussed above, establishes that fluorescence and scattered light in fact distinguish between the various cell types present in bone marrow.

Appellant further argues that, even if equivalence of the analytical methods of Bentley and the other references were conceded, no motivation has been provided for combining the disclosures of the various references cited.

It is respectfully submitted that the cited prior art provides adequate motivation for practicing the claimed processes. As discussed above, Bentley clearly discloses that when classifying and counting cells in bone marrow to be used in transplantations, the presence of lipid particles in the bone marrow results in an incorrect count of total nucleated cells.

Thus, as also pointed out above, one of ordinary skill in the art performing the analytical procedures of Inami, Kim '037, Hansen and Hoffman would have been motivated by Bentley to have classified the lipid particles present in the marrow sample so as to ensure an accurate cell count. This analysis uses only the disclosures of the cited references, and does not require any hindsight reasoning. In sum, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the appellant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

Appellant further argues that Bentley does not disclose the limitation in claim 2 which requires classifying erythroid cells into at least two groups according to the maturity of the erythroid cells. However, it is respectfully pointed out again that Bentley is relied upon in combination with a number of references which demonstrate the capabilities of the known techniques used in the claimed methods. Thus, for example, Kim '037 (e.g., column 4, lines 51-59) demonstrates that fragile blast cells and dead cells may be detected using the claimed

combination of scattered light and fluorescence. The method of claim 2 cannot therefore be considered distinguishable from the cited prior art.

Lastly, appellant urges that because claim 11 is limited to side scattered light, and that because side scattered light can reflect the intracellular information of the cells such as the nuclear forms well, this demonstrates that the aperture impedance analytical technique of Bentley is not the equivalent of the side scattering technique recited in the claims. However, it is again pointed out that Bentley is relied upon in combination with a number of references which disclose the advantages of the various cell detection methodologies recited in the claims under examination. For example, Inami (e.g. Figs. 2-11) clearly discloses the desirability of using side-scattered light, in combination with fluorescent intensity, to differentiate between the various cell types one of ordinary skill would reasonably expect to be present in a bone marrow sample.

In sum, Inami, Kim '037, Hansen, Hoffman and Kim '695 demonstrate that the claimed dyes and buffer/lysis systems were all known to the artisan of ordinary skill to be useful in the analysis of blood cell-containing samples, such as bone marrow, using the claimed analytical parameters of fluorescent and light

scattering intensity, as recited in the claims under examination. Although Inami, Kim '037, Hansen, Hoffman and Kim '695, fail to disclose the step of classifying the lipid particles present in the analyzed marrow sample as part of the step of analysis by fluorescence and scattered light, Bentley clearly discloses that when classifying and counting cells in bone marrow to be used in transplantations, the presence of lipid particles in the bone marrow results in an incorrect count of total nucleated cells. Thus, one of ordinary skill applying the analytical methods of Inami, Kim '037, Hansen, Hoffman and Kim '695 to bone marrow would have been motivated to have classified the lipid particles according to the disclosure of Bentley, so as to ensure that an accurate cell count would be obtained. It is therefore respectfully submitted that the holding of obviousness should be maintained.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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FCP
July 29, 2004

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